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CORRESPONDENCE

Elevated HbF Labelled as LA1C/cHb1 on BioRad D10 HPLC: Missed Diagnosis of Homozygous Beta Thalassemia

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Accurate and timely diagnosis, genetic counselling and prenatal diagnosis form the basis of control of thalassemias [1]. High performance liquid chromatography (HPLC) is a rapid, reproducible and accurate technique for the diagnosis of inherited hemoglobin disorders [2]. The Bio-Rad D10 hemoglobin testing system is an automated cation exchange HPLC instrument for screening of inherited hemoglobin disorders. These are diagnosed by interpretation of the chromatograms for percentages and retention time [3]. Erroneous interpretation of the peaks may lead to misdiagnosis with adverse clinical consequences. We present two patients in whom the diagnosis of β-thalassemia major was missed due to misinterpretation of HPLC chromatogram.

Case 1: An 8 months old male child was referred with severe transfusion dependent anemia. Complete blood count (CBC) showed microcytic hypochromic anemia (Table 1). HPLC done outside showed the child to be normal, mother as β thalassemia trait (HbA2-5.7%), while the father's report was not available. In view of clinical suspicion of thalassemia major, retesting was requested. HPLC at our center showed HbF-23%, HbA2-4.2% and HbA-55.9%. In view of raised HbF level and history of recent blood transfusion, thalassemia major was suspected. The outside HPLC report showed HbA1b (15.2%) and LA1C/cHb1 (69.5%) peaks, HbA2-2.8% with no HbF in the designated region. HbA0 was reported as 95.1% with no HbF (Fig. 1). The child was reported as normal on HPLC. The two prominent peaks adjacent to the HbF elution region were misinterpreted as HbA0. Since the patient had been recently transfused, the diagnosis of homozygous β thalassemia at our center was confirmed by DNA mutation study, which showed compound heterozygosity for 619 bp del and CD8/9 (+ G) mutation.

Case 2: A 2 years old girl presented with transfusion dependent severe anemia since 6 months of age and hepatosplenomegaly. CBC showed microcytic hypochromic anemia (Table 1). HPLC done outside showed HbA-96.6%, HbA2-1.8% and no HbF. The clinician at our center re-ordered HPLC as the clinical profile suggested beta thalassemia major. HPLC showed raised HbF level, which however eluted in the adjacent peaks A1b (16.6%) and LA1C/cHb1 (78.6%) with HbA2-2.7% and HbA0-1%. Outside HPLC chromatogram showed HbF elution in the adjacent peaks-A1b (17.9%) and LA1C/cHb1 (73.6%), which were misinterpreted to be HbA0 and the child was reported as normal (Fig. 1).

Accurate diagnosis of thalassemia is a key step in management and formulation of prevention strategies in the rest of the family members. HPLC plays a key role in diagnosis of haemoglobin disorders. However, like any other technique, this system has its own limitations. According to the manufacturer's guidelines on Biorad D10 system, when HbF level is $> 16.5\%$, it can elute in the adjacent windows e.g., A1b and LA1C/cHb1 and no HbF peak may be seen [4]. In our experience of 10 untransfused patients with beta thalassemia homozygosity with HbF ranging from 58.9 to 98.7%, none of them showed raised HbF peak but instead showed elution of HbF in the A1b and LA1c/CHb1 peaks. The HbA1b in these patients ranged from 12.3 to 20.6% while LA1c/CHb1 values ranged from 46.6 to 80.3%. Elution of HbF was not seen in the HbA1c region in any of these patients. Raised A1b + LA1c/CHb1 percentage is not seen in other hemoglobin

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Table 1 Red cell indices in Case 1 and 2 (done outside and at our center)

Red cell haematological	Case 1		Case 2	
Parameters	Outside	Our center	Outside	Our center
RBC ($\times 10^6$/μl)	3.01	3.45	3.3	2.78
HGB (g/dl)	5.6	7.0	6.5	5.5
MCV (fl)	69.0	64.5	66.2	65.6
MCH (pg)	18.7	20.2	19.8	18.2
RDW-CV (%)	27.8	16.7	35.0	34.8

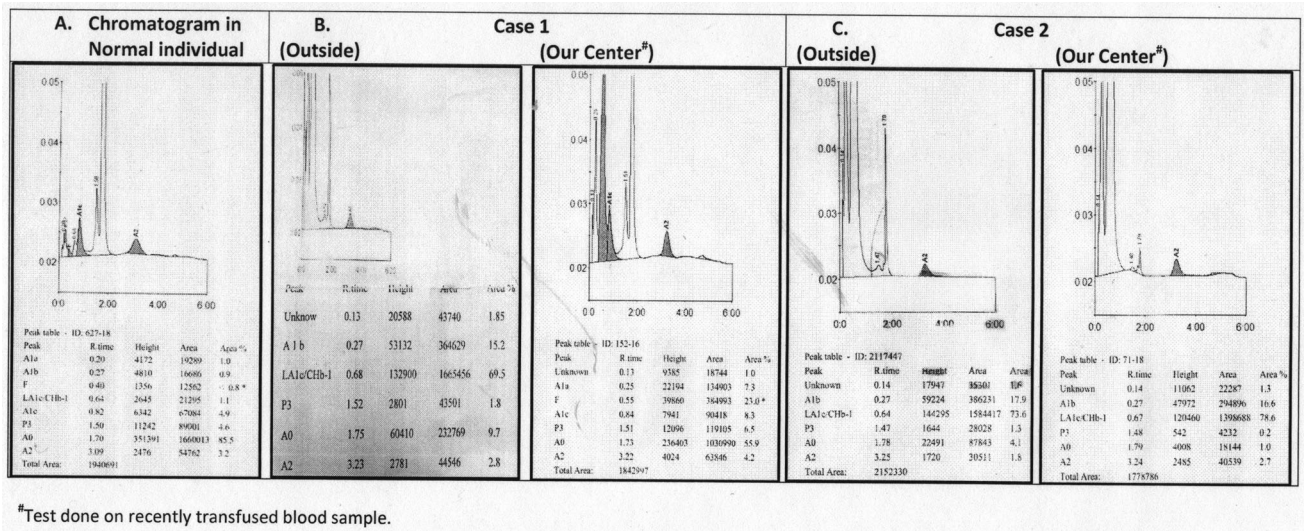

Fig. 1 HPLC chromatograms of cases of HPLC misinterpretation, reported outside and at our center on Biorad D10 Hemoglobin testing system. **a** Normal chromatogram, **b** Case 1, **c** Case 2. Note the raised A1b and LA1c/CHb1 peaks with no HbF in case 1 (outside report) and case 2 (in outside and our center's report). #Test done on recently transfused blood sample

disorders. However HbA1a may be raised in the presence of fast moving hemoglogins (HbH and HB Barts).

Accurate diagnosis requires that the cumulative percentages in the adjacent peaks (A1b and LA1C/cHb1) be considered as HbF. However, in the patients reported here, these instructions had not been followed leading to the HbF sub-fractions being overlooked and misdiagnosis of beta thalassemia major as normal. Both reports were from different outside laboratories. Such misdiagnosis can have vital implications for the management and control of thalassaemia. Patients may be inappropriately managed and family screening may be overlooked. More training of the users (Technicians and pathologists) regarding the peaks generated and possibility of co-elution of different hemoglobins is required to avoid misinterpretation.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No patient/subject identifying information has been disclosed in the manuscript. No patient/subject intervention was done and the subjects were not exposed to any risks during the study.

Informed Consent Since the study involved a retrospective review of cases from routine testing offered by the laboratory, separate informed consent was not taken for the study. "For this type of study formal consent is not required".

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